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Epidemic Coxsackie Virus Infection with Mixed Clinical Manifestations

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WEBSTER DEFINES epidemic as "common to, or affecting at the same time, many in a community" (1). Classically, epidemic infectious disease is recognized when a clinical syndrome occurs common to all affected persons. Thus, epidemics caused by measles, influenza, and smallpox viruses are readily recognized and distinguished from one another on clinical grounds. However, a single agent is not always implicated in epidemic infections with homogeneous clinical manifestations. Such situations have been encountered with increasing frequency during studies of epidemic "poliomyelitis." Johnson, Shucy, and Buescher (2) found at least seven different enteroviruses associated with paralytic and nonparalytic CNS disease occurring in Hawaii in 1958, and Johnsson (3) demonstrated a similar circumstance during epidemic CNS disease occurring in Sweden in 1952. Similarly, a number of different viruses have been associated with acute upper respiratory disease, particularly in recruits (4).

The present report describes yet another type of enterovirus epidemic in which no common characteristic clinical picture occurred. Rather, epidemic dissemination of several types of Coxsackie viruses, group B, was recognized solely by laboratory investigation of febrile disease with varied symptoms which occurred during the summer of 1960, in military personnel at Walter Reed Army Medical Center and in their families.

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MATERIALS AND METHODS

PATIENTS

Studied subjects were observed in clinics and wards of Walter Reed General Hospital by hospital staff, or were seen as outpatients by the authors. All patients were residents of Metropolitan Washington.

SPECIMENS FOR VIROLOGIC EXAMINATION

Throat washings, stools, and tissues for virus isolation were obtained as early as possible after onset of symptoms and were stored at −70 C until tested. Primary monolayers of Rhesus monkey, human epithelial carcinoma (Hep 2) cells,* and suckling mice were used for virus isolation as described previously (5). Antibodies to group B Coxsackie viruses were measured in acute and convalescent sera simultaneously using either standard tissue culture tube neutralization (6) or metabolic inhibition tests (7). This combination of virologic and immunologic techniques has in our hands yielded specific etiologic diagnoses on approximately 80% of patients tested (2, 8).

RESULTS

CLINICAL AND EPIDEMIOLOGICAL DESCRIPTION OF THE OUTBREAK

Between July 27 and October 29, 1960, 43 persons presenting with different clinical syn-

* Obtained from Microbiological Associates, Inc., Bethesda, Maryland.

TABLE 1. Clinical Diagnoses and Virologic Studies in 28 Patients with Coxsackie Virus, Group B, Infections

Patient	Onset of Symptoms	Age/Sex	Clinical Diagnosis	Specimen	Coxsackie Virus Isolated	Titer of Neutralizing Antibody in Serum	
						Acute	Convalescent (virus type)
1 E. B.	28 July	35, M	Pleurodynia, orchitis	Stool	B4	<1:10	1:80
2 E. R.	27 July	2, F	Hepangina	Throat swab	B4	ND	ND
3 P. R.	1 August	28, M	Pleurodynia	Stool	B4	<1:10	1:40
4 C. W.	29 July	6, F	Abdominal pain	Stool	B4	<1:10	1:160
5 G. K.	2 August	7, F	Abdominal pain, tonsillitis	Throat swab, stool	None	<1:10	1:320 (B3)
6 F. K.	5 August	11, M	Pleurodynia	Throat swab	B3	<1:10	1:320
7 S. K.	6 August	6, F	Pleurodynia	Throat swab	B3	<1:10	1:80
8 M. K.	6 August	9, M	Pleurodynia	Stool	B3	<1:10	1:320
9 V. K.	11 August	33, F	Pleurodynia	Stool	None	<1:10	1:160 (B
10 M. D.	9 August	6, F	Fever	Throat swab	B2	1:10	1:160
11 P. T.	13 August	22, F	Myocarditis	Stool	None	<1:10	1:640 (B4)
12 B. T.	19 August	6 days, F	Encephalomyocarditis	Brain, heart, liver, Blood, etc.	B4	<1:10	1:20
13 G. M.	14 August	47, M	Pericarditis	Stool	B5	1:20	1:80
14 Ri. R.	19 August	16, M	Pleurodynia	Stool	B4	1:160	≧1:640
15 D. R.	19 August	10, M	Pleurodynia	Stool	None	1:80	1:160 (B4)
16 M. R.	19 August	6, F	Pleurodynia	Stool	B4	1:160	≧1:640
17 B. I. R.	19 August	3, F	Pleurodynia	Stool	None	1:20	1:320 (B4)
18 G. R.	25 August	13, F	Pleurodynia	Stool	B4	1:10	1:40
19 R. R.	25 August	11, M	Pleurodynia	Stool	B4	1:20	1:40
20 J. R.	27 August	7, M	Pleurodynia	Stool	B4	<1:10	1:640
21 H. R.	27 August	46, M	Pleurodynia	Stool	B4	1:320	1:640
22 Be. R.	27 August	43, F	None	Stool	None	1:320	1:1280 (B4)
23 S. R.	27 August	5/12, F	None	Stool	B4	1:10	1:640
24 D. L.	12 September	18, F	Mild encephalitis	Stool	B5	ND	ND
				Throat wash, cerebrospinal fluid	Neg		
25 M. J.	22 September	19, F	Pleurodynia, aseptic meningitis	Stool	B5	1:64	1:512
				Throat wash, cerebrospinal fluid	Neg		
26 E. N.	25 September	45, F	Fever, headaches	Stool	None	1:40	≧1:640 (B5)
27 C. D.	8 October	23 days, F	Rash	Stool	B5	<1:10	<1:10
28 L. L.	29 October	3, F	Diarrhea	Throat swab	B2	<1:10	1:640

dromes associated with fever were studied virologically. Originally a problem of providing etiologic diagnosis for attending physicians, investigations were extended to family contacts of patients when it became apparent from laboratory observations that the common factor to many patients, irrespective of signs and symptoms, was infection with group B Coxsackie viruses. Twenty-eight such patients were ultimately identified, and are the subject of this report. No virologic diagnosis was obtained for 14 of the remaining patients, and one was shown to have infection with ECHO virus, type 4.

Infection was recognized initially in late July in two physicians from this laboratory and in members of their families. During the following 3 months the remaining patients were observed in the hospital and clinics by staff physicians of the Medical, Pediatric, Surgical and Obstetrical Departments. Patients presented to each of these specialty services on the basis of their ages or predominant symptoms. Table 1 summarizes this outbreak in chronologic fashion.

Case Reports

The following descriptions illustrate the variations in the clinical illnesses.

Pleurodynia with Orchitis: (No. 1, E. B.) A 35-year-old virologist, developed headache, anorexia, fever (102 F oral) and myalgia on July 28. On the fourth day of the illness, pleuritic chest pain was noted and persisted for a day. After a 15-day asymptomatic interval, orchitis occurred followed by unilateral atrophy. The etiologic agent implicated was Coxsackie virus, type B4.

Abdominal Pain: (No. 4, C. W.) A 6-year-old daughter of a physician, was seen by the surgical service because of generalized abdominal pains of more than 6 hours' duration. Physical examination revealed an oral temperature of 102 F and diffuse abdominal tenderness. White blood count was 15,300/mm³ with 92% neutrophils; serum amylase activity was normal. At the time of appendectomy on the day of admission, a moderate amount of amber peritoneal fluid and slightly enlarged mesenteric lymph nodes were found. Histologic examination of the appendix revealed no evidence of acute inflammation. The postoperative course was uneventful, and during this period a sibling developed clinical mumps. Patient C. W. had no serologic evidence of mumps virus infection; Coxsackie virus, type B4, was associated with her illness.

Myocarditis: (No. 11, P. T.) A 22-year-old female was admitted for right lower quadrant abdominal pain during the thirty-sixth week of pregnancy. Examination revealed a rectal temperature of 99.8 F and tenderness in the right lower quadrant and along the right lower aspect of the uterus. WBC was 14,500/mm³ with 80% polymorphonuclear cells. Within a few hours of admission labor commenced; after 4½ hours a normal infant was delivered. This infant subsequently died of fulminating myocarditis (see below). Over the next 3 days, the mother's temperature varied between 97 and 102 F. Aching of the eyes and diffuse myalgias accompanied the fever which returned to normal for one day and then spiked again to 102 F. At this time the cardiac rate was very labile; an electrocardiogram showed diffuse nonspecific ST-T wave changes which were interpreted as consistant with myocarditis. Serial chest roentgenograms taken during this interval revealed definite increase in size of the heart, although at no time was the shadow beyond normal limits. By the eighth day after onset the patient was afebrile and symptom-free. The agent incriminated was Coxsackie virus, type B4.

Neonatal Encephalomyocarditis: (No. 12, B. T.) The newborn daughter of the patient above was kept in isolation because of the mother's febrile illness. During the first 5 days of life, the baby ate well and appeared normal. At 5:30 AM on the morning of the sixth day, respiratory distress and cyanosis of sudden onset were noted. Respirations were grunting in nature and at a rate of 80/min; auscultation, however, revealed normal breath sounds. The heart tones were muffled and irregular in intensity; cardiac rhythm was regular and rapid. The liver edge was palpated 6 cm below the right costal margin. An electrocardiogram revealed a nodal tachycardia with A-V dissociation. Oxygen and digoxin were administered, but the infant's condition deteriorated, and death occurred 4 hours after distress was discovered.

Postmortem examination * revealed both lungs to be collapsed and congested. There was hemorrhage into many alveoli and a round cell interstitial infiltration. There was also evidence of aspirated formula. The heart, although grossly normal, showed, upon microscopic examination, areas in which occasional fibers were necrotic and surrounded by mononuclear cells. This lesion, while seen elsewhere in cardiac muscle, was prominent only in the

* Autopsy performed by Dr. I. Roeckel.

TABLE 2. Distribution of Virus in Organs of Fatal Neonatal Encephalomyocarditis

Coxsackie Virus, Type B4 (Patient 12)	
Organ	Virus Concentration*/ gram Tissue
Blood	50,000
Brain	30
Cerebrospinal fluid	1,000
Heart	50,000
Kidney	3,000
Liver	1,000,000
Lung	3,000
Spleen	50,000

* Tissue culture infective dose 50 ($TCID_{50}$).

interventricular septum. The portal areas of the congested liver contained infiltrates of round cells and eosinophils. The kidneys were congested but not inflamed. Examination of the nervous system revealed a cloudy cisternal fluid containing 1,800 erythrocytes and 280 WBC/mm^3 (55% lymphocytes, 45% polymorphonuclear cells). The brain was very soft, and on microscopic examination perivascular lymphocytic cuffing was found in the areas adjacent to the ventricular system. Coxsackie virus of group B type 4 was recovered from tissues taken at autopsy (Table 2).

Pericarditis: A 47-year-old male (No. 13, G. M.) experienced dull, aching substernal pain on August 14 and was hospitalized with a diagnosis of myocardial infarction. Physical examination and clinical laboratory findings were normal except for the electrocardiogram which revealed slight elevation of ST segment in leads 2, AVF and the lateral precordial leads. Serial transaminase levels remained normal, and the electrocardiogram remained unchanged during the first 5 days. On the fourth day several small yellow lesions with a red base were noted on the tonsillar pillars. On the eighth day, after a weekend at home, he returned with substernal pleuritic pain, fever, and pericardial friction rub; an electrocardiogram showed markedly elevated ST segments. Pericardial effusion developed 3 days later but resorbed without paracentesis. After 9 days the patient was afebrile, asymptomatic, and heart size was normal. Subsequent electrocardiograms revealed inversion of T waves which, 50 days after the initial symptoms, reverted to normal. Virologic examinations established concomitant infection with Coxsackie virus, group B type 5.

Aseptic Meningitis: A 19-year-old Negro female (No. 25, M. J.) developed sore throat followed by pleuritic chest and right upper quadrant pain. On the tenth day of illness throbbing headache, chills, fever (102.8 F), and nausea began, resulting in hospitalization the next day. Lumbar puncture revealed 147 cells/mm^3, of which 92% were lymphocytes. Cerebrospinal fluid was sterile and sugar was normal. Fever and symptoms abated on the fourteenth day after onset. Virologic studies indicated Coxsackie virus type B5 infection.

MINOR ILLNESSES

Fever: A 6-year-old female (No. 10, M. D.) was seen at home because of fever to 105 F. Because throat culture revealed *Hemophilus influenzae,* tetracycline therapy was administered; defervescence was gradual over a 36-hour period. Two days prior to the patient's illness a younger brother had fever and pneumonitis but was not studied virologically. Coxsackie virus type B2 was associated with this illness.

Rash: (No. 27, C. D.) A 23-day-old female, developed a macular erythematous rash associated with fever. The eruption spread from scalp to the entire body, sparing the palms and soles, and lasted 4 days. Coxsackie virus type B5 was isolated from a stool specimen, but no antibody to this virus was detected in sera

TABLE 3. Clinical Syndromes Occurring During Interval of Coxsackie Virus, Group B, Epidemic*

Symptom Complex	Number of Patients with Indicated Etiology				
	Coxsackie Group B Serotype				Other†
	2	3	4	5	
Fever	1			1	5
Herpangina			1	1	
Abdominal pain		1	1		1
Pleurodynia		4	10	1	2
Orchitis			1		
Myocarditis			2		
Pericarditis				1	
Meningitis or encephalitis			1	2	5
Rash				1	1
Diarrhea	1				
No symptoms			2		

* Five patients showed more than one clinical symptom and are listed more than once in this table.

† ECHO virus, Type 4, associated with aseptic meningitis in one patient; no etiologic agent established for the remainder.

obtained on the first and eighteenth days of illness.

Diarrhea: A 3-year-old female, (No. 28, L. L.), developed fever, diarrhea, and abdominal discomfort on October 29. Physical examination showed abdominal distension and tenderness. No pathogenic bacteria were found in the stool, however, a beta hemolytic streptococcus was isolated from a throat swab, and penicillin was administered. Coxsackie type B2 infection was found.

CHARACTERISTICS OF THE CLINICAL ILLNESSES

Ten distinct syndromes as well as asymptomatic infection were associated with the 28 patients with Coxsackie virus infectious (Table 3). Many of the same clinical pictures were observed in the 14 patients without virologic diagnosis. ECHO virus type 4 infection was present in one patient with meningitis. In the group with Coxsackie virus infection, pleurodynia, or milder febrile disease with chest pain, was the most common illness, occurring in 15 of the patients. One person with pleurodynia later developed orchitis; another was shown to have meningitis. Fever alone occurred in two patients, one a child, (No. 10, M. D.), the other an adult, (No. 26, E. N.). Oropharyngeal lesions similar to those seen in herpangina were noted in two patients (No. 2, E. R. and No. 13, G. M.). A presenting symptom of abdominal pain occurred in two patients, both children (No. 4, C. W. and No. 5, G. K.). Cardiac involvement of three types was documented in this series of patients: pericarditis with effusion (No. 13, G. M.), fatal myocarditis in a newborn (No. 12, B. T.), and mild myocarditis (No. 11, P. T.). Infections of the central nervous system occurred in three instances, meningitis (No. 25, M. J.) and encephalitis (No. 24, D. L. and No. 12, B. T.), the latter being a postmortem finding. Rash (No. 27, C. D.) and diarrhea (No. 28, L. L.) were each seen once. Two members of the R. family (Nos. 22, 23) had no symptoms.

FAMILY STUDIES

Since knowledge of illness in close contacts assists in the differential diagnosis of infectious diseases, investigations of families was undertaken when possible.

Other members of patients' families were studied clinically in five instances and, in four, virologic investigation was made as well.

Patient E. B. (Table 1) developed his symptoms several days after one of his children had a febrile illness with chest pains. In the P. R. family, a child (No. 2, E. R.) was the first member to become ill. Several days later her father (No. 3, P. R.) developed pleurodynia, and her mother suffered severe myalgias. An older sibling was asymptomatic. Although the fathers in these two households were virologists, no laboratory contact with Coxsackie viruses could be established. Thus available information suggests that disease in these two individuals was contracted from their children who were the first in the family to become ill.

Four children and their mother were studied in the K. family (Nos. 5–9). The first illness occurred on August 2 as abdominal pain and tonsillitis in one of the children. Three to four days later, the other three children developed pleurodynia, and five days later the mother also developed fever and chest pain. Coxsackie B3 was incriminated in this outbreak (Table 1).

The R. family (Nos. 14–23), consisting of 10 persons, demonstrated a similar chronologic sequence with adult disease occurring after the children became sick. In this family all who became ill had a similar clinical syndrome, whereas, in the P. R. family and K. family, clinical variants occurred.

The sequence of infection was reversed in the T. family (Nos. 11 and 12) in which it is apparent that the infant who developed fatal myocarditis was infected by the mother, either *in utero* or during delivery.

CORRELATION OF VIROLOGIC OBSERVATIONS AND DISEASE

Table 1 outlines the virus isolations and serologic results for the entire group.

Twenty-one strains of group B Coxsackie viruses were recovered from the 28 persons tested; these viruses were types 2, 3, 4, and 5. Twenty-six of 28 patients were tested for antibodies to Coxsackie viruses, group B. Significant increases occurred in all but 4 of the 26.

Etiologic associations of viruses with disease were made, first, by recovery of virus from secretions or excretions and demonstration of increasing serum antibody titers during disease, (15 patients) or, second, by demonstration of antibody response alone, (6 patients) or, third, by recovery of virus from organs obtained at necropsy (1 patient). These criteria for virologic diagnosis are widely accepted, and in light of present knowledge, the minimum standards required. However, fulfilling these criteria is not always possible, particularly when efforts to obtain specific diagnoses are not made until late after onset of disease, or circumstances preclude follow-up bleedings of recovered patients. Even in such circumstances critical appraisal of antecedent medical history and epidemiologic associations suggests that illness might be caused by the agents in question. There were 4 such instances in this study (Patients #2, 15, 19, and 21, Table 1). The illnesses in these individuals occurred in families at a time when specific Coxsackie viruses were known to have been disseminated. Thus while serological evidence of concomitant infection with recovered viruses was lacking or inconclusive in these cases, it is highly probable that observed illness was indeed caused by Coxsackie viruses. On the other hand, no such assumption can be made for Patient 27 (in whom no serological response was demonstrated to recovered virus, Table 1), since evidence for antecedent or succeeding infection in the family was not obtained. Similarly, since no serologic studies were performed in Case #24 isolation of virus from the stool cannot be interpreted definitively.

Discussion

Comparable laboratory study failed to implicate Coxsackie or other enteroviruses as the cause of disease in 14 patients with illnesses similar to those seen in the other 28. This suggests that other, as yet unidentified, agents may cause pleurodynia, summer exanthems, and undifferentiated febrile illnesses. A similar situation has been shown for aseptic meningitis (8) and acute benign pericarditis (9). If these findings are substantiated by further study, the value of studying only a sample of patients to establish the etiology of epidemic infections might well be questioned.

Coxsackie viruses are rarely recovered from tissues of infected persons, except in fatal neonatal disease. The present experience with Patient No. 12 again documents the ease with which these viruses can be recovered from tissues and shows that at death such infants harbor enormous amounts of infective virus (Table 2). When organs obtained at autopsy were studied quantitatively an interesting pattern of virus concentrations was found. The largest amounts of virus were shown in blood, heart muscle, liver, and spleen, although every other organ tested yielded virus. The finding that the concentration of virus in the liver exceeded significantly that in whole blood may mean that active proliferation of virus was occurring in the liver, or that virus was being sequestered there. The presence of focal round cell infiltrates in the liver supports the former hypothesis and suggests that the process had been proceeding at least 48 hours. Comparable amounts of virus were found in blood, heart muscle, and spleen. The vascularity of the latter organ might well account for the concentrations of virus from it. However, the recovery of large amounts of virus from relatively avascular heart muscle suggests that multiplication was also occurring here. Since pathologic changes in this organ were less pronounced

than in liver, it might be inferred that establishment of virus in the heart followed that in the liver. Kibrick and Benirschke (10) described an infant dying of overwhelming Coxsackie virus type B4 infection in whom histopathologic evidence of hepatic involvement early in the course of the infection was obtained. In their review of 25 reported cases, however, microscopic evidence of hepatitis was present in only 67%. If these findings can be documented in additional patients, the heretofore unsuspected role of the fetal liver as a primary target organ for Coxsackie viruses in systemic infections can be more clearly defined.

A unique feature of this outbreak is the wide spectrum of illness which occurred within the brief time span of 3 months and in the absence of an epidemic on clinical grounds. Previous epidemics of group B Coxsackie viruses have documented the unusual clinical manifestations such as pericarditis and the common minor illness syndromes, but these have been far less conspicuous than aseptic meningitis or pleurodynia (11, 12). Atypical cases of Bornholm disease were noted long before the Coxsackie viruses were discovered (13, 14).

Although simultaneous dissemination of numerous enteroviruses through a community during the summer season is a well-known phenomenon, the isolation of four distinct Coxsackie B serotypes is unusual. This finding does not in itself explain the lack of a homogeneous clinical epidemic since studies in this laboratory of a large number of group B Coxsackie infections (15) do not suggest that any one serotype has organ or tissue tropism not shared by the others. Five group B Coxsackie serotypes have been associated with aseptic meningitis, pleurodynia and cardiac disease in the past. The most recently described member of the group, Coxsackie B6, is as yet an infrequently recognized human pathogen. With the advent of newer techniques for studying viruses in the laboratory, some epidemics with common clinical syndromes have been found to be due to simultaneous dissemination of multiple closely related viruses. The outbreaks of central nervous system infections referred to above (2, 3) are examples. The problem of acute respiratory disease in military recruits (4) is another. Now another epidemic situation requires consideration; that is, transmission of closely related viruses with production of disease of heterogeneous symptoms.

Summary

An outbreak of group B Coxsackie virus infections (types 2, 3, 4 and 5) is described in which the broad spectrum of clinical illness concentrated into a 3-month time interval was a unique feature. Pleurodynia occurred most frequently; nervous system and cardiac involvement were seen in several patients. Fever, exanthem, orchitis, rash, and diarrhea each occurred in one or two cases. Family studies indicated that infection was usually introduced into the family by children, although infection of a newborn was acquired from the mother.

Because of the variation in clinical symptoms the occurrence of an outbreak was recognized only by laboratory studies which revealed group B Coxsackie virus infections in the patients.

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Summario in Interlingua

In le curso del estate e autumno del anno 1960, quaranta-tres patientes con acute morbo febril esseva studiate pro evidentia de un infection viral. A parte casos de infection asymptomatic, dece distincte syndromes clinic esseva observate. Istos esseva febre de non-determinate origine, herpangina, dolores abdominal, pleurodynia, orchitis, myocarditis, pericarditis, menin-

gitis o encephalitis, eczema non-differentiate, e diarrhea. Vinti-octo subjectos habeva associate infectiones per virus Coxsackie gruppo B con serotypos 2, 3, 4, e 5. Le serie includeva un infection per virus ECHO typo 4. In 14 patientes le etiologia remaneva indeterminate. Esseva concludite que le dissemination de un numero de typos viral de intime affinitate pote producer intra un communitate particular un epidemia de heterogenee symptomatologia. Un tal situation es impossibile a recognoscer a base de datos exclusivemente clinic.

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